

Haptoglobin and serum enzymatic response to maximal exercise in relation to physical fitness

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ABSTRACT

SPITLER, DIANE L., W. CARTER ALEXANDER, G. WYCKLIFFE HOFFLER, DONALD F. DOERR, and PAUL BUCHANAN. Haptoglobin and serum enzymatic response to maximal exercise in relation to physical fitness. *Med. Sci. Sports Exerc.*, Vol. 16, No. 4, pp. 366-370, 1984. This study investigated the potential value of specific cellular responses to acute and chronic exercise as indices of physical fitness. Muscle enzymatic and hemolytic responses following a progressive cycle-ergometer test to maximal aerobic capacity were studied in 12 women and 12 men, aged 27 to 55 yr, who had been previously assigned to "high" and "low" fitness groups. Venous blood samples were obtained at rest prior to the cycle test, immediately following maximal effort, and 1, 2, 4, 6 and 24 h post exercise. The samples were analyzed for hematocrit (Hct), hemoglobin (Hb), lactate dehydrogenase (LDH), serum glutamic oxalacetic transaminase (SGOT), creatine phosphokinase (CK), CK isoenzymes, and haptoglobin (Hapt). Hemoglobin and Hct were the only variables to rise significantly ($P < 0.05$) following exercise, and at all sampling times were significantly lower in the women compared with the men; CK was constituted in the MM isoenzyme band at all times, and Hapt levels were significantly lower in high-fitness men and women as compared with low-fitness men and women. These results indicate that one progressive test to maximal aerobic capacity is not sufficient to induce significant muscle enzymatic or hemolytic stress responses. They do suggest, however, that a chronic hemolytic condition exists as a result of aerobic conditioning activity.

AEROBIC CAPACITY, CREATINE KINASE, HEMOLYSIS, SEX

Maximal aerobic capacity, as determined by maximal oxygen uptake, has been universally accepted as the primary indicator of cardiovascular fitness; both methodology of testing and interpretation of results have become standardized. As a result, definitive data can be obtained and used to establish fitness levels. However, maximal aerobic capacity describes only a limited aspect of physiological condition and response. The identification of indices of specific cellular responses to acute and chronic exercise would provide additional information. Muscle cell and red blood cell integrity are two potential variables. Serum enzymes are used, for example, as a measure of degree of cellular injury in the presence of various organic lesions. Moreover, serum enzyme activity can rise in many non-specific temporary states, including physical exertion (2,3,8,15,23,25). The release of enzymes from skeletal muscles following exercise may occur as a result

of muscle hypoxia (3,8), ischemia or necrosis (8), or other conditions that cause a change in cell membrane permeability (19,25). Shapiro et al. (25) have proposed that changes in membrane permeability of, or damage to, muscle cells after high-intensity exercise can be minimized by regular physical training.

A second possible index of cellular response to exercise is the haptoglobin (Hapt) level, which would reflect hemolysis caused by compression of the muscles during rhythmic physical activity. Levels of free-circulating Hapt decrease as the Hapt molecules combine with free hemoglobin to conserve iron. Because the measurement of free-Hapt in the serum has been used extensively to evaluate hemolytic activity post trauma and disease (5,18), measurement post exercise may provide evidence of an acute hemolytic condition. Lower resting-Hapt levels might indicate a greater degree of physical activity.

It was our intention to evaluate whether serum muscle enzymes and free-Hapt levels prior to and following a bout of maximal physical exertion are related to and might, therefore, serve as indices of physical fitness.

METHODS

Twelve women and 12 men, aged 27-55 yr, participated in the study. All subjects were screened by a medical history review, physical examination, resting 12 lead electrocardiogram (ECG), complete blood count, blood chemistry assays, pulmonary function test, and a treadmill stress test to voluntary maximal exertion using a modification of the Bruce protocol (4). The screening was conducted 1 to 4 months prior to the start of the study. Each subject signed a statement of voluntary informed consent prior to inclusion in the study.

The subjects were assigned to high fitness (above the age-specific mean fitness level) or low-fitness groups (below the age-specific mean fitness level) on the basis of their screening treadmill tests for maximal aerobic capacity (1). Six subjects were in each sex/fitness group.

The subjects' physical characteristics and treadmill data are summarized in Table 1. The men compared with the

TABLE 1. Physical characteristics of subjects and treadmill data.

	Above mean fitness		Below mean fitness	
	Women (N=6)	Men (N=6)	Women (N=6)	Men (N=6)
Height (cm)	161.1 \pm 2.94	174.7 \pm 3.02	163.5 \pm 2.98	176.2 \pm 3.14†
Weight (kg)	56.8 \pm 1.47	66.4 \pm 1.80	61.1 \pm 5.51	84.2 \pm 5.55*†
Age (yr)	31.0 \pm 2.94	40.0 \pm 3.14	39.2 \pm 6.04	42.3 \pm 5.35
HR Pre-exercise (beat·min ⁻¹)	74.5 \pm 3.51	64.0 \pm 4.98	92.8 \pm 7.43	96.0 \pm 4.53*
$\dot{V}O_2$ (l·min ⁻¹)	2.4 \pm 0.10	3.4 \pm 0.11	1.6 \pm 0.06	2.8 \pm 0.27*†
$\dot{V}O_2$ (ml·min ⁻¹ ·kg ⁻¹)	41.9 \pm 2.29	51.4 \pm 2.37	27.1 \pm 2.33	32.4 \pm 3.14*†
$\dot{V}E$ (l·min ⁻¹)	94.4 \pm 5.88	124.2 \pm 2.45	68.1 \pm 5.10	99.2 \pm 13.06*†
HR _{max} (beat·min ⁻¹)	190.2 \pm 3.88	183.5 \pm 6.29	177.3 \pm 5.92	181.3 \pm 4.20

Values are means \pm standard errors.

* $P < 0.05$ Fitness.

† $P < 0.05$ Sex.

TABLE 2. Responses to cycle ergometer test.

	Above mean fitness		Below mean fitness	
	Women (N=6)	Men (N=6)	Women (N=6)	Men (N=6)
$\dot{V}O_2$ (l·min ⁻¹)	2.2 \pm 0.13	3.3 \pm 0.16	1.6 \pm 0.09	2.7 \pm 0.29*†
$\dot{V}O_2$ (ml·min ⁻¹ ·kg ⁻¹)	39.4 \pm 3.35	49.4 \pm 3.18	22.7 \pm 2.78	32.4 \pm 4.20*†
$\dot{V}E$ (l·min ⁻¹)	87.5 \pm 6.78	129.4 \pm 10.57	74.5 \pm 5.55	90.1 \pm 10.00*†
HR _{max} (beat·min ⁻¹)	186.3 \pm 3.47	175.0 \pm 4.65	167.5 \pm 10.73	177.5 \pm 5.06

Values are means \pm standard errors.

* $P < 0.05$ Fitness.

† $P < 0.05$ Sex.

women were significantly ($P < 0.05$) taller and heavier and had greater oxygen uptake ($\dot{V}O_{2max}$) and ventilation ($\dot{V}E_{max}$) values. High-fitness subjects compared with the low-fitness group weighed less and had lower resting heart rates. Maximal heart rates did not differentiate on the basis of sex or fitness level.

Test subjects refrained from physical exercise the day prior to the test and reported to the laboratory following an overnight fast. While the subject was seated at rest (5 min), a venous blood sample was taken from the ante-cubital vein. The tourniquet was released immediately after venipuncture and 35 ml of blood was obtained by using vacuum tubes. The sample was analyzed for hematocrit (Hct) (12), hemoglobin (Hb) (12), lactate dehydrogenase (LDH) (29), serum glutamic oxalacetic transaminase (SGOT) (12), creatine phosphokinase (CK) (29), CK isoenzymes (6), and Hapt (7). Enzymes were analyzed on a duPont ACA III. Within- and between-day coefficients of variation for LDH were 1.8 and 2.1, respectively; 1.2 and 3.2 for CK; and 2.1 and 2.1 for SGOT. The subjects then consumed a meal-substitute bar and fruit juice. After resting 1 h, the subjects were weighed and physiological sensors were attached.

The progressive cycle-ergometer test to self-determined maximal aerobic capacity consisted of 5 min of seated rest, followed by a 3-min warm-up at 60 rpm, 25 W, and 2-min sequential test intervals of 25 W increments. The ECG (Frank lead system) was monitored continuously and recorded for 5 s of each minute. Blood pressure was monitored using an automatically inflated

cuff with microphone and was recorded at rest, during warm-up, and at 70% maximal heart rate (HR_{max}), as determined from the previous treadmill stress test. Expired air was collected and analyzed for volume, oxygen content, and carbon dioxide content utilizing a Beckman Medical Metabolic Cart. Ventilation, oxygen uptake, respiratory rate, and respiratory quotient were recorded each minute. Immediately following maximal effort, a second venous blood sample was taken. Additional samples were obtained 1, 2, 4, 6, and 24 h post exercise and were analyzed in the same manner as resting samples. The only attempt to control diet after the exercise test was to ask the subjects to limit intake of high-fat foods and to abstain from drinking alcoholic beverages.

Analysis of variance was utilized to test for group differences (sex and fitness) in a 2X2 design using the BMDP statistical package (11). Post-exercise values of all blood variables were compared with resting values by ANOVA across time. Relationships among the variables were analyzed by correlation. Statistical significance was established at the 0.05 level.

RESULTS

Results of the cycle-ergometer test are summarized in Table 2. The men had higher $\dot{V}O_2$ and $\dot{V}E$ values than the women and, as expected, the high fitness subjects had higher $\dot{V}O_2$ and $\dot{V}E$ than the low-fitness subjects.

All measured blood variables rose immediately after exercise and returned quickly to resting levels (Table 3

TABLE 3. Serum enzyme activity.

		Post Exercise					
	Rest	1 min	1 hr	2 hr	4 hr	6 hr	24 hr
High-fitness men							
CK (U·l ⁻¹)	158.8 ± 57.5†	182.7 ± 62.1	163.5 ± 55.1	164.3 ± 53.6	164.8 ± 52.2	173.7 ± 51.0	135.3 ± 26.5
LDH (U·l ⁻¹)	147.5 ± 16.8	174.8 ± 23.3	146.0 ± 13.8	149.5 ± 16.8	150.8 ± 11.1	146.3 ± 13.3	131.3 ± 12.6
SGOT (U·l ⁻¹)	31.2 ± 3.4	38.7 ± 4.1	32.5 ± 2.4	33.0 ± 2.9	32.3 ± 2.5	32.0 ± 3.4	29.5 ± 2.4
Hb (g·dl ⁻¹)	14.5 ± 0.3†	16.2 ± 0.3†	14.3 ± 0.2†	14.2 ± 0.3†	14.0 ± 0.3†	14.2 ± 0.2†	14.1 ± 0.3†
Hct (%)	42.8 ± 0.7†	46.8 ± 0.8†	42.0 ± 0.7†	42.0 ± 0.7†	41.3 ± 0.6†	41.5 ± 0.7†	41.7 ± 0.3†
High-fitness women							
CK (U·l ⁻¹)	97.8 ± 16.8†	117.0 ± 21.3	102.3 ± 17.4	101.0 ± 16.6	102.3 ± 17.3	103.3 ± 16.3	85.0 ± 9.4
LDH (U·l ⁻¹)	121.8 ± 12.1	152.0 ± 16.8	139.7 ± 18.1	134.3 ± 14.2	131.8 ± 14.9	131.3 ± 14.0	118.5 ± 13.1
SGOT (U·l ⁻¹)	26.7 ± 2.8	32.7 ± 2.3	30.7 ± 2.4	26.8 ± 1.1	27.5 ± 2.4	27.2 ± 2.4	25.5 ± 2.5
Hb (g·dl ⁻¹)	13.2 ± 0.4†	14.9 ± 0.4†	13.1 ± 0.3†	12.8 ± 0.3†	12.9 ± 0.3†	12.9 ± 0.2†	12.8 ± 0.3†
Hct (%)	38.3 ± 0.9†	43.2 ± 1.0†	37.8 ± 1.1†	37.3 ± 1.0†	37.3 ± 1.0†	37.0 ± 0.9†	37.8 ± 1.1†
Low-fitness men							
CK (U·l ⁻¹)	139.3 ± 26.4†	159.0 ± 32.3	140.3 ± 26.5	146.8 ± 24.6	154.2 ± 23.3	166.5 ± 22.7	142.5 ± 17.9
LDH (U·l ⁻¹)	155.7 ± 7.2	172.5 ± 12.2	146.7 ± 8.2	159.3 ± 6.5	153.5 ± 6.1	156.8 ± 6.6	154.3 ± 8.2
SGOT (U·l ⁻¹)	32.7 ± 3.2	36.0 ± 2.9	31.3 ± 3.3	33.3 ± 3.7	31.8 ± 2.3	34.3 ± 2.7	31.7 ± 2.4
Hb (g·dl ⁻¹)	14.7 ± 0.4†	16.5 ± 0.5†	14.7 ± 0.4†	14.8 ± 0.5†	14.7 ± 0.4†	14.6 ± 0.4†	14.6 ± 0.5†
Hct (%)	43.8 ± 0.9†	47.8 ± 0.9†	43.0 ± 1.0†	43.2 ± 1.0†	42.5 ± 0.9†	42.3 ± 1.0†	42.8 ± 1.1†
Low-fitness women							
CK (U·l ⁻¹)	94.8 ± 27.5†	105.8 ± 29.9	99.8 ± 28.8	100.0 ± 28.2	104.5 ± 27.9	106.2 ± 28.9	100.7 ± 30.6
LDH (U·l ⁻¹)	148.8 ± 8.9	165.8 ± 11.8	149.8 ± 10.4	160.0 ± 17.5	154.0 ± 10.1	151.7 ± 11.3	142.8 ± 11.2
SGOT (U·l ⁻¹)	29.7 ± 3.3	33.2 ± 4.4	30.8 ± 3.5	31.0 ± 3.5	31.3 ± 4.2	31.7 ± 4.3	32.8 ± 4.0
Hb (g·dl ⁻¹)	13.5 ± 0.4†	15.0 ± 0.5†	13.6 ± 0.4†	13.3 ± 0.4†	13.2 ± 0.5†	13.1 ± 0.4†	13.0 ± 0.4†
Hct (%)	40.7 ± 1.3†	44.5 ± 1.3†	40.7 ± 1.1†	39.8 ± 1.1†	39.5 ± 1.4†	39.0 ± 1.2†	39.2 ± 1.3†

Values are means ± standard errors.

† $P < 0.05$ sex.

and Figure 1); however, neither the serum enzyme levels nor Hapt values were significantly changed within individuals at any time as a result of the maximal ergometer exercise test.

At all sampling times, Hct and Hb levels were significantly lower in the women as compared with the men (Table 3). No differences on the basis of fitness were found. Changes in plasma volume to denote the effects of exercise-induced dehydration were calculated from Hb and Hct by the method of Dill and Costill (10). High-fitness women exhibited the largest decrease in plasma volume immediately post exercise (18.5%), with low-fitness men (17.3%), high-fitness men (16.8%), and low-fitness women (15.9%) following. Hematocrit levels increased significantly by 10%, and Hb by 12% immediately post exercise.

Haptoglobin levels were significantly higher in low-fitness men (225.0 mg·dl⁻¹) and women (220.8 mg·dl⁻¹) than in the high-fitness men (145.8 mg·dl⁻¹) and women (185.7 mg·dl⁻¹) at rest and following exercise (Figure 1). Serum Hapt levels increased 9.8% immediately following exercise and returned to baseline values within 1 h post-exercise.

Total CK levels were within the normal range for the assay in all groups at rest and remained constituted entirely in the MM isoenzyme band. The women had significantly lower resting CK levels than men; however, there were no other enzymatic differences between the sex groups, and no differences between fitness groups for LDH, SGOT, or CK levels at any sampling time (Table 3).

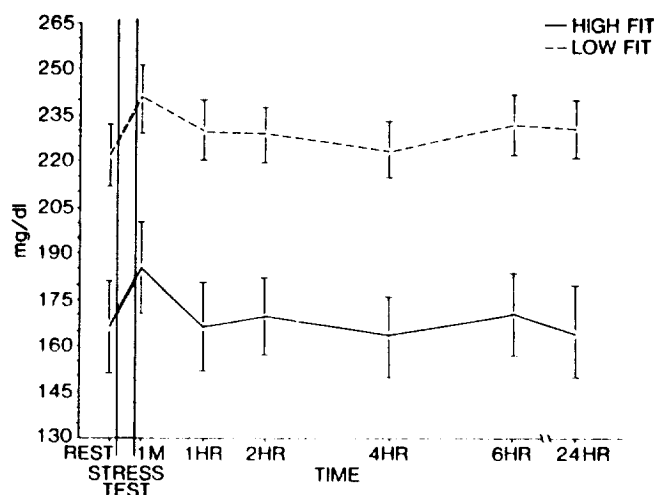


Figure 1—Changes in serum haptoglobin levels in high-fitness and low-fitness groups following cycle ergometer stress test to maximal aerobic capacity.

The correlations among the variables are presented in Table 4. Resting levels of Hapt demonstrated the greatest correlations with maximal oxygen uptake at -0.61 and were greater for the men ($r = -0.76$) than the women ($r = -0.37$).

DISCUSSION

In this investigation we evaluated serum levels of selected enzymes and free Hapt prior to and following a

TABLE 4. Oxygen uptake correlation to resting enzyme variables.

$\dot{V}O_{2max}$	CK	LDH	SGOT	
CK	0.47*			
LDH	-0.03	0.55		
SGOT	0.08	0.39	0.47	
Hapt	-0.61*	-0.33	-0.16	-0.24

* $P < 0.05$

progressive maximal exercise test in high-fitness and low-fitness subjects to determine whether an acute bout of exercise or a chronic elevation of physical activity might elicit alterations in these blood constituents.

Maximal exertion. Exercise-induced hemolysis has been described after marching (28), running (14,27), and extensive physical activity of other forms (13,17,24). The mechanisms proposed for such hemolysis are mechanical intravascular destruction of the red blood cells (9) and/or renal vasoconstriction (14). Davidson (9) suggested that any increase in mechanical trauma would exaggerate this physiological process of red blood cell destruction, implying that the degree of hemolysis would be proportional to the amount of trauma sustained. For example, running might inflict greater trauma than cycling and therefore would result in more hemolysis.

When a hemolytic state occurs, some of the hemolysis occurs intravascularly (5) which results in an elevation of free-Hb. This Hb combines with Hapt to form a complex molecule which is removed from the circulation, thus conserving iron (5,21). Circulating free-Hapt levels, therefore, also drop. If the hemolysis continues and/or exceeds the Hb-Hapt binding capacity, the reabsorbing capacity of the tubules of the kidney (16) aid in Hb conservation before any excess Hb appears in the urine. Hemoglobinuria has been the focus of much study of exercise-induced hemolysis (14,26-28). However, there may be varying degrees of exercise-induced hemolysis before the Hb-Hapt binding capacity is exceeded. Therefore, we measured circulating serum Hapt levels following maximal exercise to determine whether they might be indicators of the small differences in hemolytic activity that could be expected after strenuous exercise among persons with differing aerobic capacity. We did not find such a gradation of response in this study. Although a slight increase in serum concentration of Hapt was evident post exercise in all groups (Figure 1), this can be fully explained by the exercise-induced hemoconcentration as evidenced by calculated decreases in plasma volume of 15.9 to 18.5%. The lack of differences in Hapt response between the men and women or between fitness levels indicates that one brief bout of progressive physical activity to individual maximal intensity does not produce sufficient trauma to differentiate states of hemolysis.

While various levels of physical exercise also have been documented to cause a release of serum enzymes (CK, LDH, SGOT) it is yet a controversial phenomenon (2,15,25). A critical factor in this efflux phenomenon

seems to be the time of measurement. A variety of studies have demonstrated elevations in serum CK and other enzymes anywhere from immediately post exercise to 14 d later (15), with a peak response reported to occur between 3 and 16 h post exercise. While we found no significant changes from resting values, we did observe a peak elevation immediately post exercise for CK and the other enzymes with a return to baseline within the first hour (Table 3). This change in serum enzyme activity, however, is probably accounted for by the hemoconcentration. Four to 6 h post exercise, long after fluid redistribution should have occurred, there was another non-significant rise in CK. The true post-exercise peak may have eluded us. Individual variability in rate of enzymatic response makes quantification of time to peak response difficult when discrete sample times must be chosen.

Myhre et al. (22) suggested that LDH activity in the serum may be a better indication of intravascular hemolysis than Hapt. Increased LDH activity has been observed in patients with coronary heart disease (30) and in normal individuals following exercise (21,27). We were unable to substantiate this because the 16% increase in LDH post exercise appears to be accounted for by hemoconcentration.

SGOT has been suggested as a positive indicator of the stress of physical activity training (20); however, we found no significant changes of SGOT as a result of maximal exercise or as a factor of fitness or sex.

Physical fitness. An important finding of this research is the significantly lower Hapt levels exhibited by our high-fitness subjects throughout the study (Figure 1). These lower yet clinically normal Hapt levels could, of course, be a result of genetic disposition. However, depressed Hapt levels are consistent with the mechanical hemolysis which could occur with the regular strenuous exercise reported by our high-fitness group. While we did not quantify daily physical activity, each subject was asked not to alter his/her activity schedule between the time they were classified as high- or low-fitness and the date they were being tested. We assume, therefore, that no abrupt changes in activity outside the laboratory interfered with the protocol.

In addition to differences between fitness groups, resting Hapt levels were significantly and negatively correlated with aerobic capacity ($r = -0.61$) for the combined group of men and women. When the data were separated by sex, we found the men had a Hapt-to- $\dot{V}O_{2max}$ correlation of -0.76 and the women a correlation of only -0.37 . This is consistent with a relationship between Hapt levels and lean body mass. The relatively larger muscle mass of the men may provide a larger potential site for mechanical hemolysis. Another explanation may be due to the fact that the high fitness men in our study were more highly trained than the high fitness women. Three of the high fitness men were actively training for marathon distances, while the most fit women were running shorter

distances or involved in other activities such as competitive water skiing.

Serum enzyme activity following maximal work was only moderately related to $\dot{V}O_{2\max}$ (Table 4). These data do not agree with those studies (3,25) which reported that the serum concentration of muscle enzymes varies inversely with maximal aerobic capacity or that the trained individual compared to the untrained individual has lower post-exercise serum CK levels (15,23) with earlier peak rises (23). We were unable to confirm this and suggest that muscle enzyme release is a diffuse mechanism only adequate to assess extreme muscle trauma well beyond levels experienced in a cycle ergometer test for aerobic capacity.

In this study we have demonstrated that serum Hapt levels—but not serum levels of the enzymes CK, LDH, and SGOT—differ between high-fitness and low-fitness groups of men and women and, therefore, may serve as

an index of physical fitness. Because serum Hapt levels did not change significantly following a bout of maximal exercise, a baseline Hapt sample might serve as an indicator of regular rhythmic activity and fitness (Figure 2 and Table 4). As our high-fitness subjects were all involved in regular programs of exercise, it is possible that Hapt is an indicator of chronic activity rather than of fitness. Further research is needed on the relationship of Hapt to maximal aerobic capacity or phase of physical training.

The authors acknowledge Dr. M.A.B. Frey and Sue Loffek, The Biometrics Corporation, for their assistance in the preparation of this manuscript and Harold Reed, NASA, for technical assistance.

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